INFLUENCE OF INDIVIDUAL AMINO ACIDS ON UPTAKE AND INCORPORATION OF VALINE, GLUTAMIC ACID AND ARGININE BY UNFERTILIZED AND FERTILIZED SEA URCHIN EGGS [1]

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In the course of investigations (*cf.* Tyler, 1965a) into the mechanism of the initiation of protein synthesis by sea urchin eggs, some variable results were obtained in tests with dactinomycin (actinomycin D). This inhibitor of DNA-primed RNA synthesis stimulated incorporation of labeled valine into protein in four experiments with suspensions of eggs that contained many oocytes but failed to do so in several subsequent tests. The nutritional status of the animals, and consequently of the eggs, was considered as one possible source of this variation. Tests were therefore made of the effects of glucose, which experiments by Honig and Rabinovitz (1965) had shown could prevent or relieve dactinomycin-induced inhibition of protein synthesis in sarcoma-37 cells. However, glucose did not enable dactinomycin to enhance incorporation of amino acid into protein by sea urchin eggs. Tests were then made with mixtures of amino acids. Again no stimulation was obtained with dactinomycin on the incorporation of a labeled amino acid. In these tests another phenomenon appeared, namely, a marked inhibition by the amino acid mixture on the incorporation of the labeled amino acid. The experiments on the oocytes, including the erratic dactinomycin effect, will be reported elsewhere (Piatigorsky, Ozaki and Tyler, 1966), while the present account will deal mainly with exploration of the competition among amino acids.

That the rate of uptake of one amino acid may be inhibited by the presence of others has been shown in many experiments with intact cells of various organisms (see Wilbrandt and Rosenberg, 1961; Christensen, 1962, 1964; Johnstone and Scholefeld, 1965, for review). In general, the inhibition is found to occur between members of the same general class of amino acid and is interpreted as being due to a competition for transport across the cell surface.

Since sea urchin eggs are the subject of increasing numbers of investigations of amino acid incorporation into protein by the intact cells, it seemed to us desirable to determine whether or not such competition at the cell surface occurs with this material, too, and if so, to examine the interrelationships among the amino acids. While this work was in progress a preprint was received of an article by Mitchison and Cummins (1966) concerning the uptake of labeled valine and cytidine by sea

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urchin eggs at various stages of development. In tests with eggs at one hour after fertilization they report a marked inhibition of the uptake of C^{14}-valine by each of five neutral amino acids (L-leucine, DL-isoleucine, DL-alanine, DL-phenylalanine, DL-threonine) and slight inhibition by one basic amino acid (DL-lysine).

Our experiments show that these findings hold also for unfertilized eggs and provide further evidence, from measurements of both accumulation and subsequent incorporation of amino acid into protein, supporting that of Mitchison and Cummins that the inhibition operates as a competition for entrance into the cell. We have extended the measurements to include all twenty of the "coded" amino acids tested, with both fertilized and unfertilized eggs, for ability to inhibit both uptake and incorporation into protein of a neutral (valine), acidic (glutamic acid) and basic (arginine) amino acid. In the present article the amino acids that are termed basic are histidine, arginine and lysine. The acidic group includes aspartic acid, glutamic acid and their derivatives asparagine and glutamine. The remaining thirteen of the "coded" amino acids are placed in the neutral group.

Materials and Methods

Eggs were obtained from the sea urchin *Lytechinus pictus* by the method of KCl-injection, the suspension temporarily acidified to pH 5 to remove the gelatinous coat, and an aliquot removed for counting (Tyler and Tyler, 1966). For the tests of uptake and incorporation the eggs were incubated with the C^{14}-labeled amino acid and the C^{12}-amino acid being explored, in a total volume of 0.25 ml. of artificial sea water, at pH 8.0, in polystyrene test tubes, for the specified time and at 20° C. At the end of the incubation period a large excess (1 ml. of an ice-cold 0.1 *M* solution) of the C^{12}-amino acid, corresponding to the C^{14}-amino acid, was added as quencher. For the measurements of uptake the eggs were thoroughly washed with ice-cold artificial sea water and transferred with distilled water to filter papers which were rapidly dried and placed directly in the scintillation fluid [4] in which radioactivity was determined (Tri-Carb spectrometer) with about 50% efficiency. For the measurements of incorporation the same filter papers were rehydrated by transfer through absolute alcohol, 95% alcohol and 5% trichloroacetic acid (TCA). They were then processed, as usual (Tyler, 1966), with hot TCA, the alcohols, and ether, and transferred to the vials of scintillation fluid for determination of incorporation of the labeled amino acid into protein.

Results

1. *Inhibition of uptake of C^{14}-valine by an amino acid mixture*

As indicated above the initial experiments on the effect of additional amino acids on the incorporation of labeled valine were done in connection with tests of the action of dactinomycin. Table I gives the results of two experiments in which the incorporation of C^{14}-valine into protein was measured in the presence and absence of a mixture of amino acids (Borsook *et al.*, 1957) with and without dactinomycin. The inhibiting effect of the amino acid mixture is marked, regardless of whether or not dactinomycin is present. The latter had no significant effect on C^{14}-valine

[4] 2.88 g. PPO (2,5-diphenyloxazole) and 0.34 g. dimethyl POPOP (1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene) per liter of toluene.

TABLE I

Action of an L-amino acid mixture and of dactinomycin** on incorporation of C^{14}-valine into protein by unfertilized eggs of L. pictus, incubated for 30 minutes at 20° C.*

Experiment	C^{14}-valine (sp. act. 185 C./M) μc./ml.	Counts per minute per 10^4 eggs			
		Without dactinomycin		With 0.015 mg./ml. dactinomycin	
		Without amino acid mixture	With amino acid mixture	Without amino acid mixture	With amino acid mixture
1	0.50	8327 9226	216 284	10519 12112	178 173
2	0.42	1050 1701	27 24	1078 1313	14 22

* Composition and final concentrations in mmoles/l.: Alanine, 0.33; arginine, 0.08; aspartic acid, 0.48; cysteine, 0.06; glutamine, 1.33; glycine, 0.89; histidine, 0.40; isoleucine, 0.05; leucine, 0.67; lysine, 0.30; methionine, 0.06; phenyl alanine, 0.26; proline, 0.23; serine, 0.28; threonine, 0.28; tryptophan, 0.05; tyrosine, 0.14.

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incorporation in these experiments. Differences between the two experiments in the absolute values for incorporation of C^{14}-valine may reflect differences in size of the endogenous free valine pool in the eggs.

The presence of the added amino acids did not, then, enable the eggs to show a stimulated incorporation of C^{14}-valine in response to dactinomycin, that had been previously noted with some batches of eggs of *Lytechinus* (see introduction).

2. *Pretreatment with amino acids*

In order to determine whether the inhibiting effect of the additional amino acids is on the accumulation of valine by the eggs or on its subsequent incorporation

TABLE II

Effect of pretreatment with an amino acid mixture (a.a. mix.) on the uptake of C^{14}-valine by unfertilized eggs of L. pictus, incubated for 1 hour at 20° C.*

Pretreatment for 1 hour in:	Counts per minute per 10^4 eggs			
	Total uptake		Incorporation into material precipitable by 5% trichloro-acetic acid	
	In presence of a.a. mix.	In absence of a.a. mix.	In presence of a.a. mix.	In absence of a.a. mix.
s.w.	80 100	23470 27714	4 10	580 638
a.a. mix.	94 132	26442 27774	8 6	1332 1338

* 0.53 μc./ml.; sp. act. 185 c./M.

into protein, tests were made on eggs that had been pretreated with the amino acid mixture and washed just before addition of the C^{14}-valine. Table II gives the results of one such experiment. The total uptake of C^{14}-valine, as well as the incorporation into protein, were determined. As the data show, preliminary exposure of the eggs to the mixture of amino acids has no effect on the subsequent uptake of the C^{14}-valine, either in the presence or in the absence of the C^{12}-amino acid mixture. But, uptake is almost completely suppressed by the amino acid mixture present during the period of incubation with the C^{14}-valine. The effect on uptake can, then, account for the inhibition of incorporation in the experiments shown in Table I.

The data of Table II also show inhibition of incorporation into protein in those eggs concurrently exposed to the amino acid mixture, regardless of prior exposure to the amino acid mixture. Furthermore there is an apparent increase in incorporation by those eggs exposed to the amino acid mixture before incubation with the C^{14}-valine alone. In three additional experiments an increase was obtained in one, while no appreciable difference was observed in the other two. At present, then, there is no consistent evidence that preincubation with other amino acids results in an increased incorporation of C^{14}-valine into protein.

Despite the washing following the pretreatment period the eggs probably retain most of the accumulated amino acids. This seems clear from experiments of other investigators (Nakano and Monroy, 1958; Mitchison and Cummins, 1966) and is indicated here by the large quantity of acid-soluble radioactivity remaining in the washed eggs. One may conclude, then, that retained amino acids do not influence the uptake of another amino acid, namely, valine. One or more of the amino acids in the added mixture evidently inhibit the uptake of valine when concurrently present in the medium. This was explored further with the individual amino acids and with fertilized as well as with unfertilized eggs.

3. *Effect of one amino acid on the uptake and incorporation of another*

(a) *C^{14}-valine*

Uptake, and incorporation into protein, of C^{14}-valine, C^{14}-glutamic acid and C^{14}-arginine by unfertilized and fertilized eggs were measured individually in the presence of an excess (*ca.* 3000 ×) of each of the other 19 "coded" amino acids. In some experiments the labeled amino acid was tested against the other 19 amino acids at the same time. In others about half of the amino acids were tested at one time, as noted in the legends for the figures. The results are represented graphically in Figures 1, 2 and 3. Tables III and IV present ratios of the average uptake of the labeled amino acid in the presence of the added C^{12}-amino acid to that in its absence. Ratios for incorporation are similarly presented. Table III includes results of an additional series of tests of incorporation (see footnote to table). In Figures 1, 2 and 3, for each experiment, the control values (indicated by NONE) are given at the top. These are followed by the values obtained for each of the added amino acids arranged in a decreasing (using the larger of each of the duplicate values) order of uptake.

For the unfertilized eggs the two experiments of Figure 1 with C^{14}-valine show marked (greater than 50%) inhibition of uptake by SER, ARG, ASN, GLN, ALA,

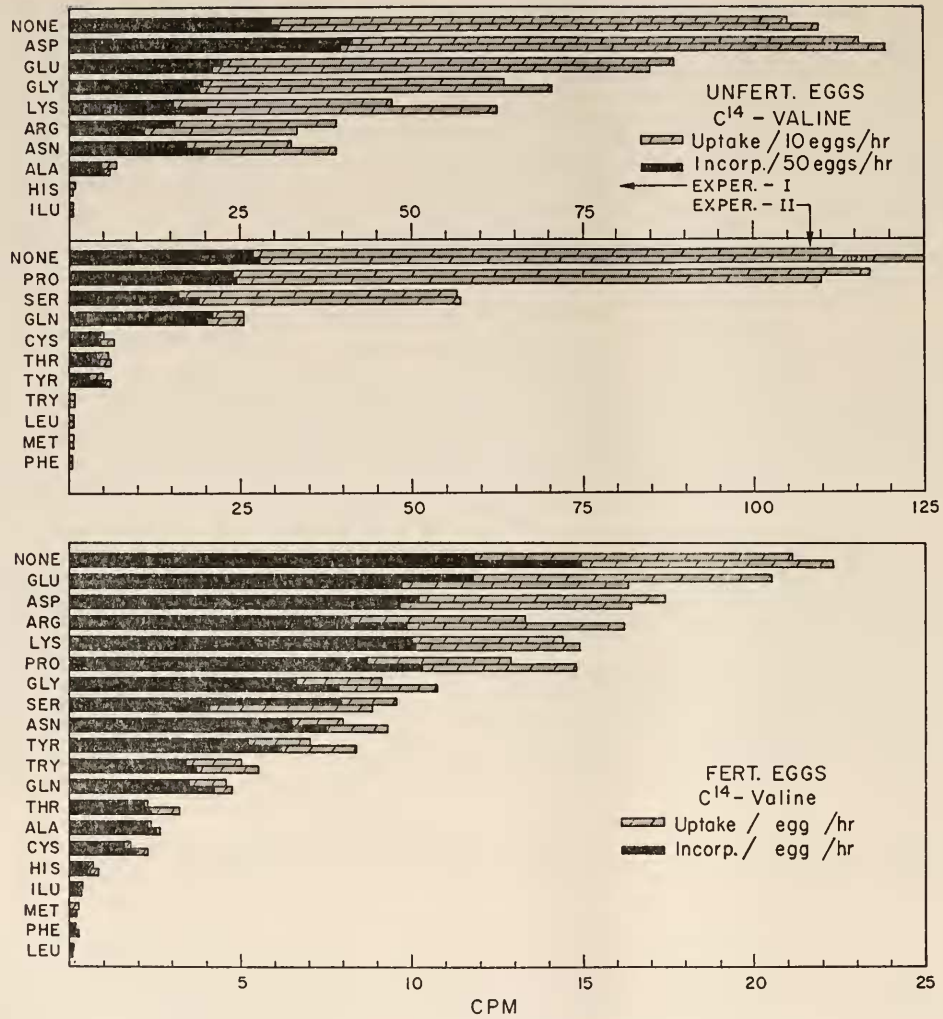

FIGURE 1. C^{14}-L-valine uptake, and incorporation into protein, by unfertilized and fertilized (one hour after fert.) eggs of *L. pictus* in presence of various individual C^{12}-L-amino acids. Incubation was for one hour in a total volume of 0.25 ml. of artificial sea water containing, per tube, 940 eggs (unfert., expt. I), 2860 eggs (unfert., expt. II) or 4314 eggs (fert.), and 0.83 μc./ml. of the C^{14}-valine (sp. act. 208.5 c./*M*). The added amino acids were each at 0.012 *M* except TYR which was at 0.0004 *M*. The tests were all run in duplicate and the individual results are represented by each member of the pairs of bars. For the unfertilized eggs the tests were done with 9 of the C^{12}-amino acids (expt. I) on one day and with the remaining 10 (expt. II) on another occasion, using eggs from a different female. In the experiment with the fertilized eggs all 19 of the C^{12}-amino acids were tested at one time. For the entries to the left of the figure: None = no added C^{12}-amino acid; ALA = alanine; ARG = arginine; ASN = asparagine; ASP = aspartic acid; CYS = cysteine; GLU = glutamic acid; GLN = glutamine; GLY = glycine; HIS = histidine; ILU = isoleucine; LEU = leucine; LYS = lysine; MET = methionine; PHE = phenylalanine; PRO = proline; SER = serine; THR = threonine; TRY = tryptophan; TYR = tyrosine; VAL = valine.

CYS, THR, TYR, HIS, ILU, LEU, MET, PHE, TRY, listed in decreasing order of the average values, as given in Table III. For all except the first three of these the inhibition of uptake is greater than 75%, and for all except the first four the inhibition of uptake is greater than 90%. The values for degree of inhibition of incorporation are similar to those for inhibition of uptake for each of the tested amino acids except for GLN where incorporation is much less inhibited (24 to 30%) than is uptake (79%).

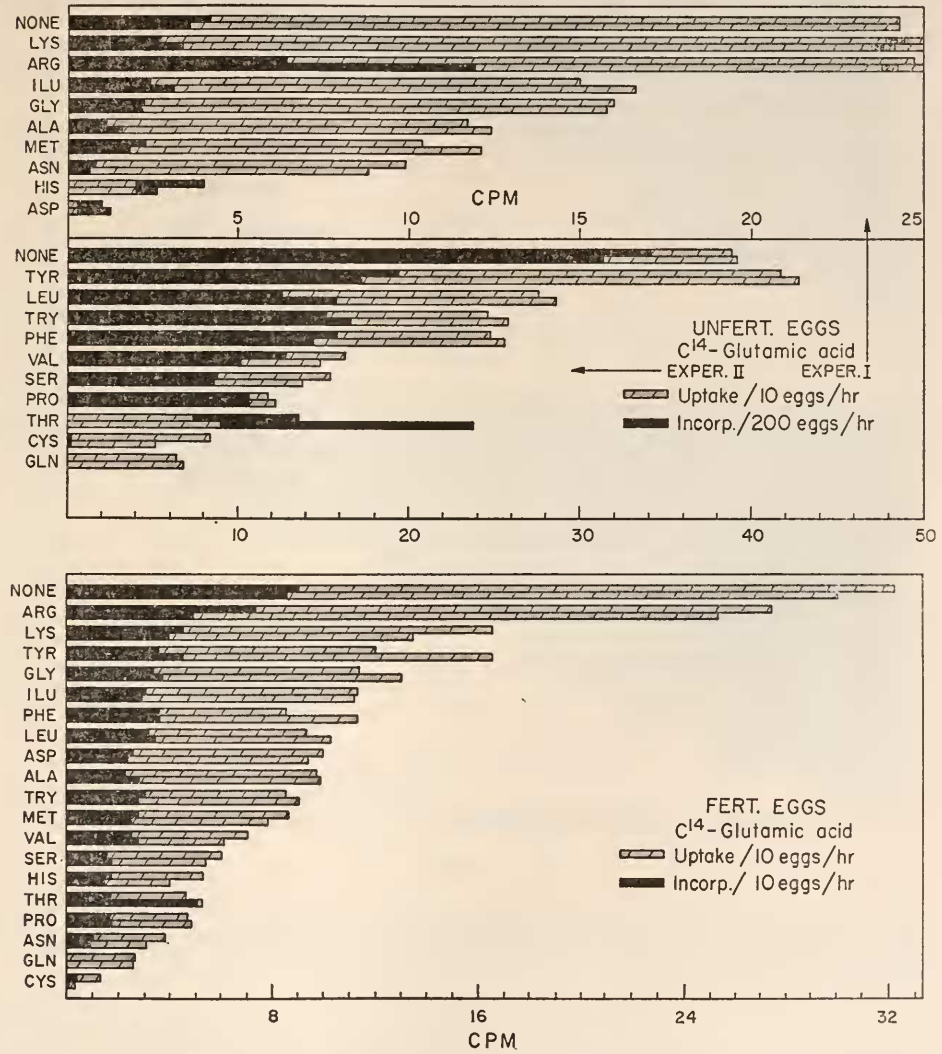

FIGURE 2. C^{14}-L-glutamic acid uptake and incorporation into protein; same description as for Figure 1, except that egg numbers were 3650 (unfert., expt. I), 1570 (unfert., expt. II) and 1190 (fert.).

For the fertilized eggs the amino acids that effect greater than 50% inhibition of both uptake and incorporation are the same as for the unfertilized eggs, except that the following are now brought just within this group: GLN (incorp.), GLY (uptake) and SER (incorp.). At the 75% level of inhibition the same amino acids

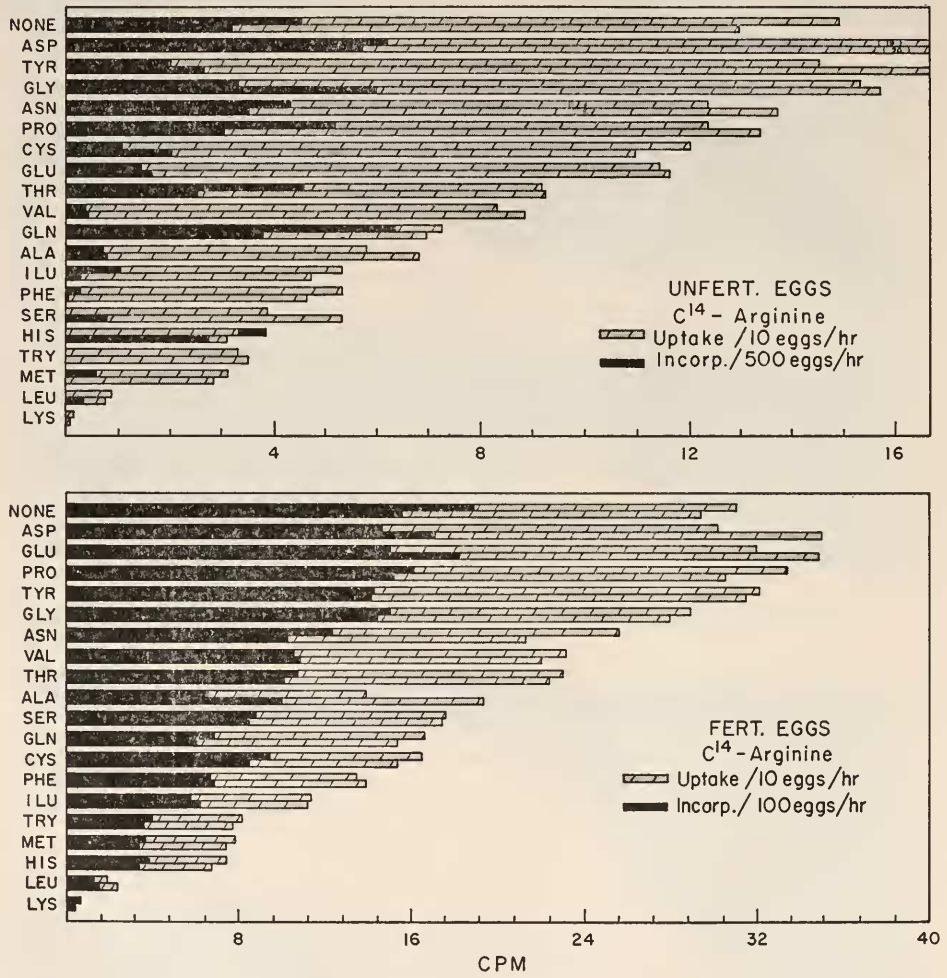

FIGURE 3. C^{14}-L-arginine uptake and incorporation into protein; same description as for Figure 1, except that egg numbers were 4170 (unfert.) and 4140 (fert.), that *L. anamesus* instead of *L. pictus* was used in the experiment with the unfertilized eggs and that the sp. act. of the C^{14}-arginine was 222 c./*M*.

are effective except for TYR (uptake and incorp.) and TRY (incorp.). Even at the 90% level of inhibition most of the inhibiting amino acids are the same as for the unfertilized eggs with respect both to uptake and incorporation, as comparisons of the values in Tables III and IV show.

The amino acids that effect the high (90% or better) degree of inhibition of uptake of C^{14}-valine, both for unfertilized (ALA, CYS, THR, TYR, HIS, ILU, LEU, MET, PHE and TRY) and for fertilized (CYS, HIS, ILU, LEU, MET and PHE) eggs all belong to the neutral group, with the exception of HIS which is only weakly basic. This holds also for the inhibition of incorporation into protein.

TABLE III

Influence of individual amino acids on the uptake and incorporation into protein of a neutral, an acidic and a basic amino acid by unfertilized eggs of Lytechinus pictus, incubated for 1 hour at 20° C.*

"Competing" C^{12}-amino acid at 0.012 *M*	Ratios of cpm's for mixture of C^{12}- and C^{14}-amino acid to cpm's for C^{14}-amino acid alone								
	C^{14}-L-Valine (3.9×10^{-6} *M*)			C^{14}-L-Glutamic acid (3.9×10^{-6} *M*)			C^{14}-L-Arginine (3.7×10^{-6} *M*)		
	Total uptake	Incorp.	Incorp.**	Total uptake	Incorp.	Incorp.**	Total uptake	Incorp.	Incorp.**
Alanine	0.07	0.17	0.08	0.49	0.34	0.42	0.45	0.22	0.35
Arginine	0.34	0.44	0.54	1.07	2.36	1.31	—	—	—
Asparagine	0.33	0.64	0.83	0.04	0.20	0.09	0.93	1.07	0.70
Aspartic acid	1.09	1.35	0.92	0.01	0.29	0.12	1.38	1.65	1.94
Cysteine	0.05	0.15	0.01	0.18	0.07	0.69	0.81	0.47	0.83
Glutamic acid	0.80	0.71	0.81	—	—	—	0.83	0.43	0.95
Glutamine	0.21	0.76	0.70	0.17	0.00	0.20	0.51	1.42	0.90
Glycine	0.62	0.64	0.80	0.65	0.55	0.49	1.11	1.32	0.68
Histidine	0.00	0.01	0.46	0.09	0.85	0.26	0.23	0.92	0.39
Isoleucine	0.00	0.01	0.02	0.65	0.70	0.47	0.36	0.19	0.14
Leucine	0.00	0.01	0.01	0.72	0.44	0.55	0.06	0.07	0.09
Lysine	0.51	0.58	0.60	1.12	0.79	0.51	0.01	0.35	0.06
Methionine	0.00	0.02	0.01	0.46	0.52	0.26	0.22	0.07	0.01
Phenylalanine	0.00	0.01	0.01	0.65	0.46	1.15	0.35	0.15	0.34
Proline	0.95	0.89	0.89	0.31	0.33	0.52	0.93	1.15	0.87
Serine	0.48	0.63	0.71	0.37	0.27	1.13	0.33	0.08	0.23
Threonine	0.05	0.17	0.13	0.21	0.57	0.72	0.66	1.00	0.46
Tryptophan	0.00	0.03	0.04	0.64	0.49	0.52	0.24	0.00	0.20
Tyrosine***	0.05	0.15	0.12	1.08	0.56	0.66	1.12	0.65	0.68
Valine	—	—	—	0.40	0.35	0.53	0.62	0.10	0.39

* *Lytechinus anamesus* used in experiments with C^{14}-L-arginine, columns 1 and 2. Eggs of this species resemble closely those of *L. pictus*.

** Separate experiment in which only incorporation into protein was measured.

*** At 0.0004 *M*.

(b) *C^{14}-Glutamic acid*

For the unfertilized eggs all but five (ARG, GLY, ILU, LYS and TYR) of the 19 C^{12}-amino acids cause greater than 50% inhibition of uptake or incorporation, or both, of the C^{14}-glutamic acid. With the fertilized eggs all but one (ARG) do so. The 75% (or more) inhibition level with unfertilized eggs is attained by ASN, ASP (uptake), CYS, GLN, HIS (uptake), and THR (uptake). At this level, for the fertilized eggs, these same amino acids, except for ASP, are effective as are also HIS (incorp.), PRO, SER, THR (uptake) and VAL (uptake). At the 90%

level with the unfertilized eggs there are ASN (uptake), ASP (uptake), CYS (incorp.) and GLN (incorp.). With the fertilized eggs 90% inhibition is given only by CYS and GLN.

For the inhibition of uptake and incorporation of C^{14}-glutamic acid there again appears to be a relationship to type of amino acid. Thus strong inhibition is given by ASP, ASN and GLN which are all grouped in the acidic category. Only CYS and THR, of the neutrals, and HIS, of the basics, strongly inhibit uptake by the

TABLE IV

Influence of individual amino acids on the uptake and incorporation into protein of a neutral, an acidic and a basic amino acid by fertilized eggs of Lytechinus pictus, 1 hour after fertilization. Incubation was for 1 hour at 20° C.

"Competing" C^{12}-amino acid at 0.012 *M*	Ratios of cpm's for mixture of C^{12}- and C^{14}-amino acid to cpm's for C^{14}-amino acid alone					
	C^{14}-L-Valine (3.9×10^{-6} *M*)		C^{14}-L-Glutamic acid (3.9×10^{-6} *M*)		C^{14}-L-Arginine (3.7×10^{-6} *M*)	
	Total uptake	Incorporation	Total uptake	Incorporation	Total uptake	Incorporation
Alanine	0.11	0.18	0.32	0.29	0.55	0.48
Arginine	0.68	0.67	0.85	0.68	—	—
Asparagine	0.40	0.52	0.11	0.11	0.78	0.65
Aspartic acid	0.78	0.74	0.31	0.27	1.08	0.92
Cysteine	0.09	0.14	0.03	0.04	0.53	0.52
Glutamic acid	0.85	0.81	—	—	1.11	0.97
Glutamine	0.21	0.29	0.08	0.01	0.53	0.38
Glycine	0.46	0.54	0.39	0.38	0.94	0.85
Histidine	0.04	0.05	0.15	0.17	0.24	0.21
Isoleucine	0.02	0.02	0.37	0.33	0.38	0.36
Leucine	0.01	0.01	0.32	0.37	0.08	0.08
Lysine	0.67	0.75	0.48	0.48	0.02	0.03
Methionine	0.01	0.02	0.27	0.30	0.25	0.23
Phenylalanine	0.01	0.01	0.32	0.38	0.45	0.40
Proline	0.64	0.71	0.16	0.21	1.06	0.91
Serine	0.42	0.43	0.18	0.20	0.58	0.50
Threonine	0.13	0.17	0.16	0.38	0.75	0.61
Tryptophan	0.24	0.27	0.29	0.33	0.26	0.21
Tyrosine*	0.36	0.43	0.46	0.46	1.05	0.83
Valine	—	—	0.21	0.30	0.75	0.62

* At 0.0004 *M*.

unfertilized eggs, and these same amino acids plus PRO, SER and VAL are similarly effective with the fertilized eggs.

(c) *C^{14}-Arginine*

Inhibition greater than 50%, for the uptake and incorporation, or both, of C^{14}-arginine, was obtained with all of the added C^{12}-amino acids with the exception of ASN, ASP, GLU, GLY, PRO, THR and TYR for the unfertilized eggs and, in addition, CYS and VAL for the fertilized eggs. At the 75% level of inhibition, only ALA, HIS, ILU, LEU, LYS, MET, PHE, SER, TRY and VAL for the

unfertilized eggs and HIS, LEU, LYS, MET and TRY for the fertilized remain inhibitory. At the 90% level of inhibition of uptake and/or incorporation LEU, LYS, MET, SER, TRY and VAL remain for the unfertilized eggs while only LEU and LYS are effective in the fertilized eggs.

It is evident that amino acids categorized as acidic did not significantly inhibit the uptake of C^{14}-arginine. In fact, ASP showed a slight enhancement of uptake and incorporation for the unfertilized eggs but this effect was not repeated with the fertilized eggs. Only a few neutral amino acids appreciably inhibited C^{14}-arginine uptake. On the other hand, both basic amino acids, LYS and HIS, showed strong inhibition for both unfertilized and fertilized eggs.

4. *Effect of fertilization on uptake of amino acids and on incorporation into protein*

Apart from the inhibitory effects of added amino acids, the data of Figures 1, 2 and 3 also permit incorporation to be compared with uptake with regard to the changes they undergo upon fertilization for C^{14}-valine, C^{14}-glutamic acid and C^{14}-arginine. This information is summarized in Table V. It is clear that uptake of

TABLE V

Effect of fertilization on uptake of amino acids and on incorporation into protein by eggs of Lytechinus (from data of Figures 1, 2 and 3; average values of cpm's per 10^3 eggs for 1 hour incubation)

	C^{14}-Valine			C^{14}-Glutamic acid			C^{14}-Arginine		
	Uptake (U)	Incorp. (I)	I/U	Uptake (U)	Incorp. (I)	I/U	Uptake (U)	Incorp. (I)	I/U
Unfertilized	11361	576	0.05	3172	91.7	0.03	1398	7.2	0.005
Fertilized	21738	13398	0.62	3116	901	0.29	3029	173	0.057
Fert./Unfert.	1.91	23	12.4	0.98	9.8	9.67	2.17	24	11.4

all three of these amino acids is high in the unfertilized egg. Upon fertilization there is an approximately two-fold increase in uptake of valine and of arginine, and no appreciable change in uptake of glutamic acid, at the stated external concentrations. The data for incorporation, however, show the usual great stimulation that occurs upon fertilization. In the present experiments these amount to 23- to 24-fold for valine and arginine, and 10-fold for glutamic acid. If incorporation is expressed in terms of uptake (columns 4, 7 and 10 of Table V) then the increase upon fertilization is of the order of 10-fold for all three amino acids, at the indicated concentrations and incubation time.

These comparisons are made apart from considerations of possible feedback inhibition of uptake, particularly in the fertilized eggs, and of possible effect of depletion of labeled amino acid from the medium. The data of Mitchison and Cummins (1966) show that with C^{14}-valine at a concentration of 0.14 mM there is no appreciable feedback inhibition of uptake by fertilized sea urchin eggs during a period of one hour. The concentration of valine in the present tests (0.0039 mM) is very much less than this. Therefore, feedback inhibition is unlikely. While similar information is not available for glutamic acid and for arginine the present

data would indicate that feedback inhibition is not likely to have occurred to any very appreciable extent in these experiments.

With regard to depletion of the labeled amino acid from the medium, calculations from the data presented in Figures 1, 2 and 3 show that the average concentrations in the medium at the end of the incubation period are reduced by approximately 2% for glutamic acid, 5% for arginine and 40% for valine. It is only for valine, then, that the value for uptake by the fertilized eggs may be appreciably affected by depletion of the label. The 40% reduction by the end of the incubation period would mean an approximately 20% average decrease in uptake, assuming linearity between uptake and concentration. This does not require altering the above statement of an approximately two-fold increase upon fertilization.

The external concentration employed in tests of valine-uptake is about one-fifth that found by Mitchison and Cummins (1966) to give maximum rate of uptake with fertilized eggs of *Paracentrotus lividus*. These workers, using concentrations well above that giving maximum rate of uptake for fertilized eggs, report a considerable increase in uptake upon fertilization. This may be estimated from their Figure 1 to amount to 15- to 30-fold. It would appear, then, that the amino acid concentrations at which the present measurements were made were in a range at which the uptake rate relative to the maximum attainable for the unfertilized egg was higher than that for the fertilized egg. This may also mean that the maximum rate is reached at lower concentrations for unfertilized than for fertilized eggs.

Discussion

The present results provide information of use in studies of changes in protein synthesis upon fertilization and early development of sea urchin eggs. The demonstration by Mitchison and Cummins (1966), with fertilized sea urchin eggs, of the ability of one amino acid to inhibit the accumulation of another, has been confirmed, and the tests have been extended to include all twenty of the "coded" amino acids in the presence of a characteristic neutral, acidic and basic amino acid in both unfertilized and fertilized eggs. The analysis has shown that competition occurs primarily between amino acids that belong to the same group. However, these interrelationships are not exclusive and there is a certain degree of overlap.

As noted in the introduction there have been many studies (*e.g.*, Wilbrandt and Rosenberg, 1961; Scholefeld, 1961; Jacquez, 1961a, 1961b; Christensen, 1962, 1964; Christensen *et al.*, 1962; Oxender and Christensen, 1963; Johnstone and Scholefeld, 1965; Guroff *et al.*, 1964; Larsen *et al.*, 1964; Spencer and Brody, 1964; Adamson *et al.*, 1966; Alvarado, 1966) with cells of various other kinds of organisms, in which the influence of one amino acid on the uptake of another has been examined. Competition is found to occur largely within the separate groups but there are many exceptions. The same general conclusions apply to the results of our experiments.

The concentration of the competing amino acid in each of our tests with valine is many thousands of times higher than that at which, according to Mitchison and Cummins (1966), the maximum rate of uptake is attained. This is probably true also for glutamic acid and arginine although the plateau levels for these have not been determined. We may infer, then, that the experiments reveal all instances in which a particular amino acid has some appreciable ability to compete for entrance

into the cell with the three amino acids tested. The correlations between the uptake of an amino acid and the incorporation into protein are very good for the unfertilized eggs and even better for the fertilized eggs where the values are higher and variation is correspondingly lower. Thus, the inhibition that one amino acid effects on the incorporation of another evidently takes place at the uptake site. That this site operates independently of the sites of protein synthesis is suggested by the wide divergences between uptake and incorporation with respect to the changes in these properties that are observed upon fertilization.

As noted above, and as is summarized in Table V, the unfertilized eggs exhibit a relatively high capacity for uptake of the three test amino acids, and the increase upon fertilization is evidently rather small. The high amino acid uptake rate of the unfertilized egg contrasts with other uptake systems studied in sea urchin eggs. For example, phosphate uptake (Whiteley, 1949; Whiteley and Chambers, 1961) and nucleoside uptake (Nemer, 1962; Piatigorsky and Whiteley, 1965; Mitchison and Cummins, 1966) are very strongly suppressed, as is the transport of many other substances in the unfertilized sea urchin egg (*cf.* Monroy, 1965; Rothschild, 1956).

Apart from the theoretical considerations that are of interest in the transport of amino acids into cells, one may utilize the data presented here, in combination with measurements of the maximum rates at which the various labeled amino acids are incorporated into protein, to specify the more effective mixtures of amino acids for labeling nascent protein in sea urchin eggs. Measurements of rates of incorporation of individual labeled amino acids as a function of concentration have been made on eggs of *Lytechinus* at one hour after fertilization (Tyler, 1965b and unpublished). From these measurements the presently available values for the (approximately) maximum incorporation into protein, in mμmoles incorporated in one hour by 10^4 eggs (and the values for the external concentrations, in μmoles/ml., at which maximum or near maximum incorporation is first attained given in parentheses) are as follows: ALA-2.3(30), ARG-2(>60), ASN-0.5(2.8), ASP-1.7(>1.9), CYS-CYS-0.3(sat. in s.w.), GLU-4.5(40), GLN-2.6(0.1), GLY-1(3.8), HIS-0.5(0.1), ILU-3.8(0.3), LEU-1.6(0.03), LYS-0.5(120), MET-0.2(0.03), PHE-0.4(0.1), PRO-1(>0.24), SER-2(8), THR-2.5(1.0), TRY-2(sat. in s.w.), TYR-0.2(0.4), and VAL-3.3(0.1).

Depending upon how the various parameters are evaluated and combined a number of highly effective mixtures may be formulated. The general procedure is to maximize incorporation into protein while minimizing effects of competition among the amino acids. It is assumed that the labeled amino acids would be available at about the same specific activity. One example of a group of amino acids that would yield high radioactivity of nascent protein is: ILU, ARG, GLU and PRO. The addition of other amino acids would tend to reduce incorporation by virtue of competition of uptake. However, one may substitute for each of these certain other "competing" amino acids that have reasonably high values of incorporation when tested individually. For example, if VAL were substituted for ILU there would not be a very great over-all change in the values for incorporation given by the mixture. Similarly ASP could be substituted for GLU without large effect, as could LYS for ARG. Obviously, there are many more complex mixtures and substitutions that might be formulated, but since the present tests were made

with only 57 of the 380 possible combinations, further assessment of the most effective mixtures does not seem warranted at this time.

Summary

1. Tests were made of the uptake and incorporation into protein of a neutral (C^{14}-valine), an acidic (C^{14}-glutamic acid) and a basic (C^{14}-arginine) amino acid in the presence of a mixture of other amino acids and in the presence of a great excess (3000-fold) of each of the other "coded" amino acids by unfertilized and fertilized eggs of *Lytechinus pictus*.

2. The results showed competition occurring principally among amino acids belonging to the same group. For C^{14}-valine the amino acids that effected strong inhibition (90% or greater) of uptake with unfertilized eggs were ALA, CYS, THR, TYR, HIS, ILU, LEU, MET, PHE and TRY, and with fertilized eggs were CYS, HIS, ILU, LEU, MET and PHE. For C^{14}-glutamic acid 90% inhibition of uptake was given by ASN and ASP with unfertilized eggs and by CYS and GLN with fertilized eggs. Finally, strong inhibition of C^{14}-arginine uptake was demonstrated by LYS and LEU with both unfertilized and fertilized eggs. Similar results were obtained in the corresponding tests of incorporation into protein. The inhibitory effects on incorporation are, then, attributable to competition for uptake.

3. In contrast to the relatively low capability of the unfertilized egg to incorporate amino acid into protein it possesses a relatively high ability to accumulate amino acids from the surroundings. For C^{14}-valine and C^{14}-arginine, the uptake rate by the unfertilized egg was approximately half of that of the fertilized egg, while for C^{14}-glutamic acid the pre- and post-fertilization rates of uptake were approximately the same.

4. The percentage of accumulated C^{14}-amino acid that was incorporated in one hour into protein in these experiments with valine, glutamic acid and arginine was 5, 3 and 0.5, respectively, in the unfertilized eggs and 60, 30 and 6, respectively, in the fertilized eggs. When expressed in terms of uptake, and assuming no large change in the pool of free amino acid in the egg, there is an approximately 10-fold increase in incorporation into protein upon fertilization for each of these three amino acids.

5. The results, also, enable formulations to be made of the kinds of combinations of labeled amino acids that would be the more highly effective in labeling nascent proteins of sea urchin eggs. One such combination would be ILU, ARG, GLU and PRO with each of these being replaceable by certain alternative "competing" amino acids as indicated in the text.

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